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Effect of Adding Iron on Nutrient Consumption and Determining the Causative Factor for Growth in the Marine Waters of the Syrian Coast

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ABSTRACT

This research aims to study the effect of Fe fertilization on phytoplankton growth and nutrient consumption in some areas of the Syrian coast. The experiment was conducted during the period from February 1 to February 21, 2021, and samples were collected from the river al Kabir al Shamali Estuary and the Higher Institute for Marine Research and transferred to the laboratories of the Higher Institute for Marine Research. As the water was enriched with Fe and nutrients, samples were taken periodically with 2-3 days interval for nutrient and chlorophyll a measurements, the experiment spanned for 21 day. Iron played an important role in stimulating growth, as the results showed that Fe-treated samples recorded the highest values of phytoplankton growth and nutrient electrolyte consumption compared to the rest of the samples. The results showed that Fe concentrations of 10 μmol/L were sufficient to reach the maximum growth of phytoplankton, as we did not observe obvious differences in growth compared to the concentration of 100 μmol/L. Phytoplankton showed a clear growth ability in the two studied stations in the Fe-treated samples, where growth continued throughout the experiment period and a jump in growth occurred on the twelfth day of the experiment and nutrients remained available in the medium until the end of the experiment, while the growth of phytoplankton was less in the control medium where Phosphate in the control samples in the marine research area played the role of the limiting factor for growth, but in the downstream region, growth continued until the end of the experiment, and there was no nutrient ion depletion with a jump in growth on the second day. The application and investment of the results of this type of experiments on various environmental fields and fish farms with economic and environmental returns is important.

Keywords: Fe fertilization, Primary productivity, phytoplankton growth, Nutrient utilization.

1. INTRODUCTION

The seas and oceans cover about 70% of the Earth's surface, so they occupy 96% of the biosphere, It plays a vital role in controlling the global climate, as well as in

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providing the natural resources needed by man (Planavsky et al., 2021.) Nutrients are defined as the group of chemical elements required for the synthesis of living organic matter they are phytoplankton growth restricting agents and include dissolved inorganic electrolytes of nitrogen, phosphorous and silicon (Prasad et al., 2021). In addition to these nutrients, there are other elements indispensable to the growth of phytoplankton, such as trace minerals (Sunda, 2012), Several studies have indicated that iron is a vital nutrient for the growth of phytoplankton (Tagliabue et al., 2017). iron is normally transported to the marine environmental through three pathways: rivers input, atmospheric deposition, seafloor processes such as decomposition and resuspension of sediments, and hydrothermal mixing (Sholkovitz et al., 2010, Elrod et al., 2008, Chase et al., 2005.)

The importance of phytoplankton lies in the fact that they are the basis of the food chain and spread in the surface layer of water or the luminous layer. These organisms produce oxygen and food through the process of photosynthesis, forming chlorophyll (Fleurence, 2021). Iron as a mineral is known to play important roles in the growth of phytoplankton in the oceans (Tripathy and Jena, 2019), Recent studies conducted in certain areas of the seas and oceans indicated the important role of Fe in the growth of marine plants, especially in temperate regions (Martin et al., 1994). There is no doubt that the total iron concentrations in coastal areas in general are several times greater than the values of iron concentrations in the open ocean, as the role of Fe in limiting primary production in coastal areas seems unlikely, However, several studies conducted in coastal areas have indicated that Fe can limit temporal growth in the California coast (Bruland et al., 2001)As well as in some areas of the fjord, such as the Trondheim Strait in Norway (Öztürk et al., 2002). Among all the trace minerals, Fe is particularly effective in stimulating biochemical activities (Shcolnick and Keren, 2006, Morel and Price, 2003.) It is an effective agent that controls the growth of phytoplankton and limits their growth in up to 40% of ocean waters (Zhao et al., 2018), Photosynthesis may be the largest Fe aggregation site within the phytoplankton cell (Strzepek and Harrison, 2004). The bioavailability of iron has far-reaching implications for many natural systems because Fe is one of the most important mineral elements in the metabolic reactions of phytoplankton (Larson et al., 2015). It is necessary for building the reactions of many proteins and enzymes (Ishizaka et al., 2005.)

Importance and objectives of the research

This study is complementary to other studies on the Syrian coast, which will shed light on the effect of Fe on the formation of primary products in the food chain and know the extent of their relationship to the nutrients available in our marine waters, which has not yet been studied in detail, as Fe plays an important role in controlling primary productivity because the growth of Phytoplankton is determined by the abundance of Fe in many areas of global waters. Fe deficiency impedes the biological use of available nutrients and also affects the quantitative and qualitative composition of phytoplankton in these areas. Accordingly, there is an urgent need to conduct such a study in Syrian marine waters in order to assess the role of Fe fortification of marine waters and its impact on primary productivity in coastal waters in Lattakia, as it lacks such studies.

2. MATERIALS AND METHODS

Study locations

The study was conducted on the marine waters of the beach of Lattakia on two terminal: the river al Kabir al Shamali Estuary (35°76'19.1"N; 35°54'19.3"E), which represents an open area affected by agricultural and industrial activities and sewage channels, the second terminal of the Higher Institute for Marine Research (35°35'31.5"N 35°44'32.2"E), which represents an area relatively far from external sources. The samples were collected from the coastal surface waters from the two studied sites by means of 25 liter pre-cleaned polyethylene containers during the winter (February) of 2021. Field measurements (pH, temperature and water salinity) were taken and the water was transferred directly to the laboratories of the Higher Institute of Marine Research at Tishreen University to conduct the laboratory study.

Preparation of Fe and Nutrient Enrichment Experiments

The water was transferred to the laboratories of the Higher Institute for Marine Research and distributed into four preparations, then distributed in polyethylene containers, as follows (control samples, Fe 10, Fe 100, nutrients). The first section is sea water that does not contain any additives (control samples). While the second section (Fe 10) is a seawater sample with Fe added at a concentration of 10 μmol/L nitrate at a concentration of 200 μmol/L ammonia at a concentration of 200 μmol/L silicate at a concentration of 2 mmol/L phosphate at a concentration of 100 μmol/L ،While the third section (Fe 100) is a seawater sample with Fe added in a concentration 100 μmol/L and nitrates, ammonia, silicates and phosphates in the same concentrations as the previous nutrients in the second section, As for the fourth section (nutrients), it is a sample of seawater to which (nitrates, ammonia, silicates, and phosphates were added, and with the same concentrations of nutrients as before in the second preparation, but without the presence of Fe .

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Deionized water was used to prepare the solutions used in the experiment, where Fe was added in the form of a solution of Fe(III) chloride salts ($FeCL_3$) and nitrates in the form of a solution of ($NaNO_3$) and silicates in the form of a solution of (Na_2SiO_3..H_2O), and ammonium in the form of a solution of (NH_4CL) and phosphates in the form of a solution of (KH_2PO_4).

Marine waters were incubated for 21 days in the open air and periodic samples were taken according to the following schedule {1, 2, 5, 7, 12, 15, 21} days.

Approved method Grasshoff (Grasshoff et al., 2009)to determine the concentration of ammonium ions in sea water, which is based on the reaction of ammonia with hypochlorite in an alkaline medium to give monochloramine, which in turn reacts with phenol in the presence of an excess amount of hypochlorite, forming indophenol blue, which absorbs light at wavelength 630 nm. The standard method for determining dissolved nitrite ions in seawater, according to Robinson and Benchneider, is based on the reaction of nitrite with sulfonylamide hydrochloride to form diazonium, which binds with [n-(1-naphthyl)-ethylenediamine dihydrochloride], which leads to the formation of a pigment. The azo that absorbs light at a wavelength of 540 nm (Grasshoff et al., 2009).

The concentration of nitrate ions was determined using the same method for determination of nitrite by reducing nitrate ions to nitrate using a copper-clad cadmium column (Grasshoff et al., 2009). Approved method (Grasshoff et al., 2009)to determine the concentration of inorganic phosphorous ions based on the reaction of ammonium molybdate with phosphate ions in the presence of triple antimony as an intermediate to obtain phosphomolybdate acid, the complex produced by ascorbic acid is reduced to give phosphomolybdenum blue, which absorbs light at a wavelength of 885 nm. To determine the concentration of dissolved silicate ions in water, the Korolev method was adopted, which is based on the formation of silicomolybdic acid when acidified samples are treated with molybdate solution, which is due to the silicomolebdic blue complex, mediated by ascorbic acid and in the presence of oxalic acid. This bench absorbs light at a wavelength of 810 nm (Grasshoff et al., 2009).

To determine the concentrations of chlorophyll pigments in water, the Jeffrey and Humphrey method was used(Jeffrey and Humphrey, 1975,) Where the samples were filtered on cellulosic filters, and then crushed by means of a vertical mixer. Chlorophyll a was extracted from the samples in the dark by 90% acetone, then the absorbance of the samples was measured by a spectrophotometer and the concentration of chlorophyll a was determined using mathematical equations (Jeffrey and Humphrey, 1975.)

Water salinity and temperature were determined using a branded field meter (WTW-Multi 340 i). All required absorbance measurements were carried out using a spectrophotometer, ZUZI brand (4211/20 Models).

3. RESULTS AND DISCUSSION

The temperatures during the experiment stages ranged between 16-19 °C, the highest recorded on the seventh day of the experiment and the lowest on the second day in all the preparations, as the preparations were subjected to the same conditions .The study indicated (Jia et al., 2022)temperature plays an important role in the growth of phytoplankton.

The pH values of the preparations ranged between 7.2-8.1, the lowest recorded at the beginning of the experiment in the Fe preparations because the added Fe was prepared with an acidic solution. The pH values increased with the passage of the incubation time in all the preparations, as the photosynthesis process consumes carbon dioxide (CO_2) from the medium (Ferderer et al., 2022).

The salinity percentage during the incubation period of the water samples ranged between 11-36‰, the highest value was recorded in the marine research area, and the lowest was recorded in the downstream region. Fresh River with salt sea water.

The initial concentrations of nutrients ranged in the estuary region river al Kabir al Shamali Estuary (k) for nitrates, phosphates, silicates and ammonia (132.631, 5.758, 176.465, 57.382 μmol/L) in sequence (Table 1) While the initial concentrations of nutrients in the marine research area (M) were nitrate, phosphate, silicates and ammonia (12.138, 0.631, 16.762, 0.927 μmol/L) in sequence (Table 1). These values converged with the values recorded in many previous local studies conducted on these two regions (Krawi *et al.*, 2013, Darwich and Sulaiman, 2013).

The difference in the concentrations of nutrients mentioned above in the two sites is due to the spatial differences, as they were high in the al Kabir al Shamali Estuary area as a result of the river water inputs that drain the nutrients from the agricultural lands. Compared to the HIMR area, which is a relatively remote area from external sources of nutrients. The low concentration of nutrients in summer is attributed to their consumption by phytoplankton to build biomass that is active in summer as a result of high temperatures and several hours of illumination, as many studies confirmed the important role that temperature and light play in the process of photosynthesis (Noiri et al., 2005.)

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Table (1): Initial concentrations of chlorophyll a and nutrients at al Kabir al Shamali Estuary stations (K) and Marine Research (M).

Station	Chl a ($mg.m^{-3}$)	NO_3^- (µmol/L)	PO_4^{-3} µmol/L)(	SiO_4^{-4} (µmol/L)	NH_4^+ (µmol/L)
K	2.728	52.631	5.758	176.465	57.382
M	0.595	19.138	0.631	36.762	0.927

Phosphate in the control samples in the marine research area played the role of the limiting factor for growth, as the increase in chlorophyll a concentrations continued, accompanied by a decrease in nutrient concentrations, until phosphate was depleted from the medium on the twelfth day of the experiment, and then the biomass began to decline in the control samples, but in the downstream area it continued Growth until the end of the experiment was not observed depletion of any mole of nutrient electrolytes (figure)1 and (figure 2). This is consistent with many studies that have shown that phytoplankton continues to grow in the event of nutrients availability and its growth declines after the nutrients are depleted from the medium (Darwich and Sulaiman, 2013, Umezawa et al., 2022).

On the first day of the experiment (d1), the concentrations of all nutrients were maximum (Figure 1) and (Figure 2), then the concentrations of nutrients began to decrease during the 21-day experiment, as (Table 2) shows the difference between the initial concentrations of the elements Nutrients (nitrate, phosphate, silicate, ammonia) and the final concentrations in all preparations On the second day of the incubation period (d 2) the amount of consumption of nutrients was low in the four preparations (Fe 10, Fe 100, nutrients, control) due to the fact that the experiment in its beginning, Where the biomass declines as a result of the shock that occurred to it as a result of changing environmental conditions during its transfer to the laboratory and the different sources of nutrition and the different sources of lighting, where many studies referred to this stage of the experiment (Boyd et al., 2000, Mohamed and Amil, 2015).

Table (2): Total change in chlorophyll A and nutrients concentrations within the preparations (control, Fe 10, Fe 100, nutrients) at the al Kabir al Shamali Estuary (K) and Marine Research (M) stations.

Station	Treatment	ΔChl a $mg.m^{-3}$	Δ NO_3^- µmol	Δ PO_4^{-3} µmol	Δ SiO_4^{-4} µmol	Δ NH_4^+ µmol
K	**Control**	4.593	30.931	3.387	89.265	41.982
	Fe 10	96.025	82.516	12.613	348.766	76.482
	Fe 100	95.926	78.523	13.128	346.056	79.782
	nutrients	48.604	49.125	8.282	306.265	59.882
M	**Control**	1.641	8.278	0.631	26.362	0.807
	Fe 10	45.546	47.711	6.558	210.562	51.725
	Fe 100	44.772	43.049	7.243	213.862	52.207
	nutrients	32.001	27.849	3.658	85.262	34.6

The amount of nutrients decreased on the fifth day of the experiment (d5), as the amount of nutrients consumed during the incubation period increased in all preparations. Fe preparations recorded the greatest amount of nutrient consumption, while it was lowest in the control samples, and here a breaking onset of the nutrient graphs was observed (Figure 1) and (Figure 2).

On the seventh day of the experiment (d7), the consumption of nutrients continued in all the preparations, and this stage constituted a stage similar to the fifth day in terms of the percentages of total nutrients consumption during the experiment.

On the twelfth day of the experiment (d 12) the consumption of nutrients continued in all preparations, and this stage constituted the largest proportion of the total nutrient consumption during the experiment, and this was accompanied by the

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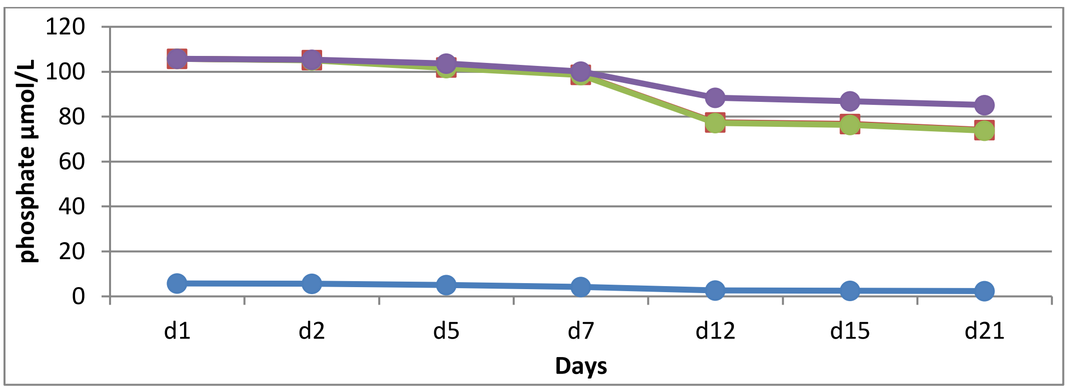
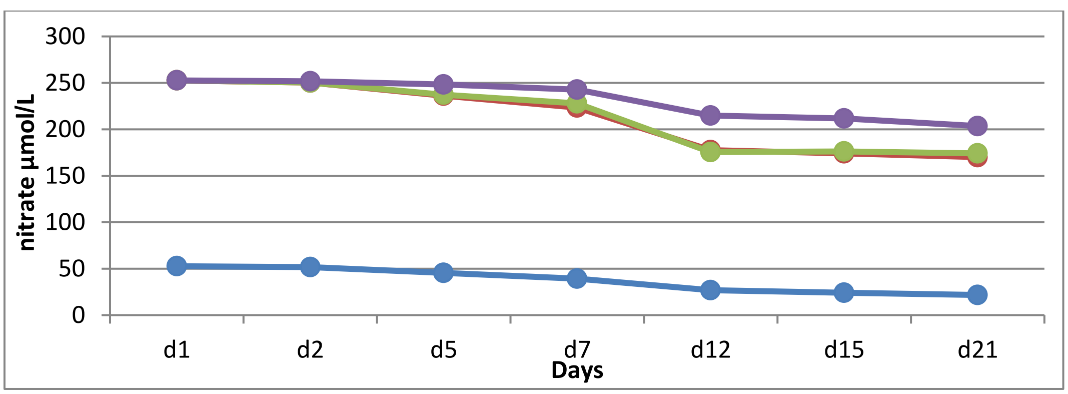
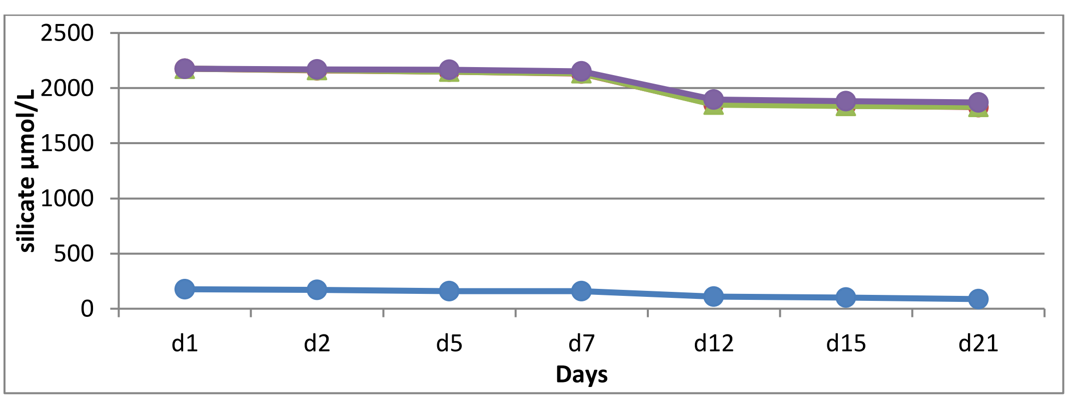
appearance of a clear peak of chlorophyll a. As shown in Figures (3, 4), but in the control sample at the marine research station, phosphate was depleted from the medium, while the rest of the nutrients continued to be present. The percentage of nutrient consumption decreased from the median on days (d 15, d 21) and this was accompanied by a decline in the growth of biomass, where the graphs of chlorophyll showed a noticeable decline in this period (Figures 3, 4). In the control sample of the marine research area, the rest of the nutrients were consumed in very small quantities after the depletion of phosphate, and this period recorded the lowest amount of consumption of nutrients during the experiment relative to the control sample.

Fe treatments recorded a greater amount of nutrient consumption compared to the nutrient treatments and control samples, where the greatest amount of nutrient consumption was in the preparation of Fe-100 from station (K) and the lowest in the control sample from station (M), where many studies indicated that Fe stimulates the growth of Fe Phytoplankton and diatoms have a great demand for nutrients in general and silicates in particular(Giri et al., 2022, Balaguer et al., 2022, Mohamed and Amil, 2015.)

The highest amount of nitrate consumption in Fe treatments was 10 in the samples from station (K) and the lowest in the control sample from station (M). The availability and consumption of the available nutrients continued until the end of the experiment and this was accompanied by a decrease in the nutrient concentration with an increase in the biomass of phytoplankton due to the activity of photosynthesis of phytoplankton(Ragueneau et al., 2002), Because nitrogen and phosphorous are essential for the growth of phytoplankton (Golterman and De Oude, 1991), While the availability of silicates is necessary for the growth of diatoms, as it is included in the construction of their siliceous shield (Tréguer and De La Rocha, 2013).







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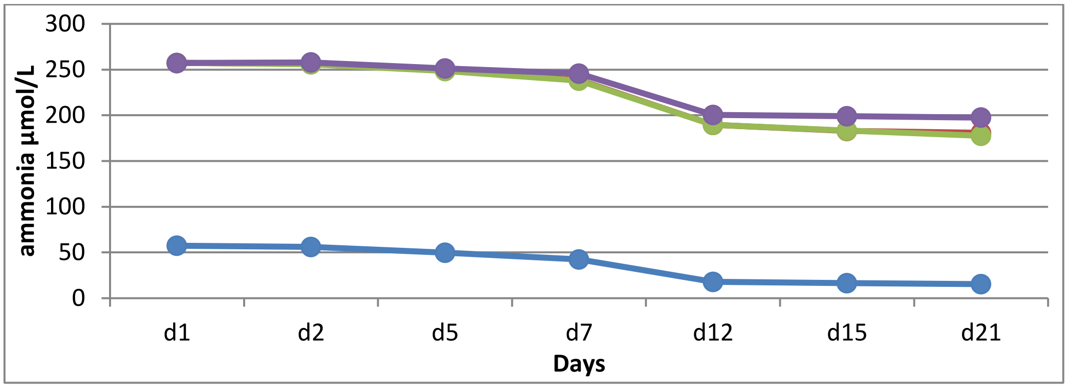
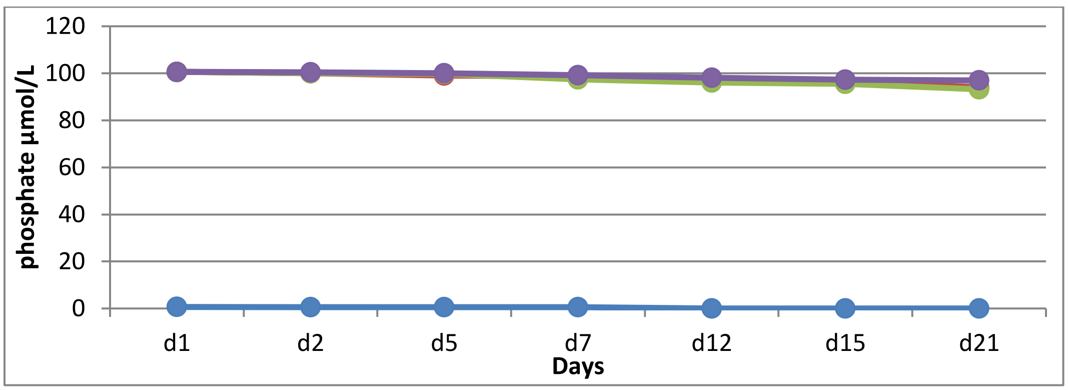
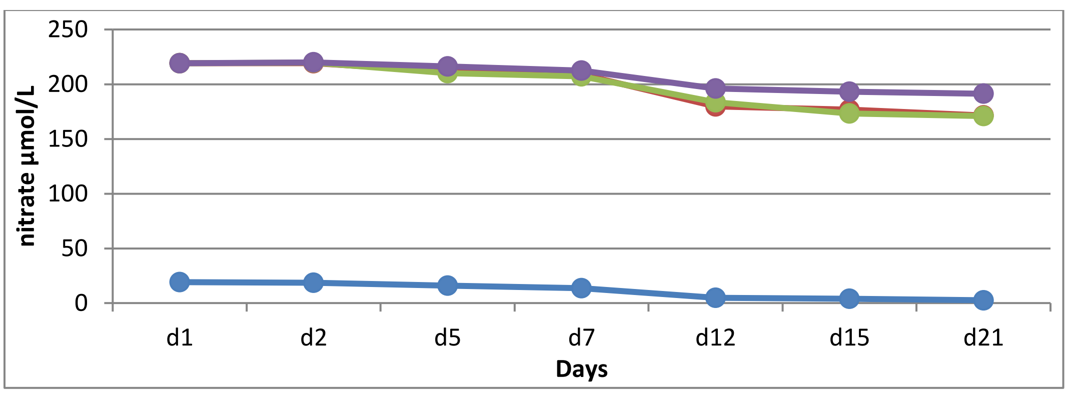
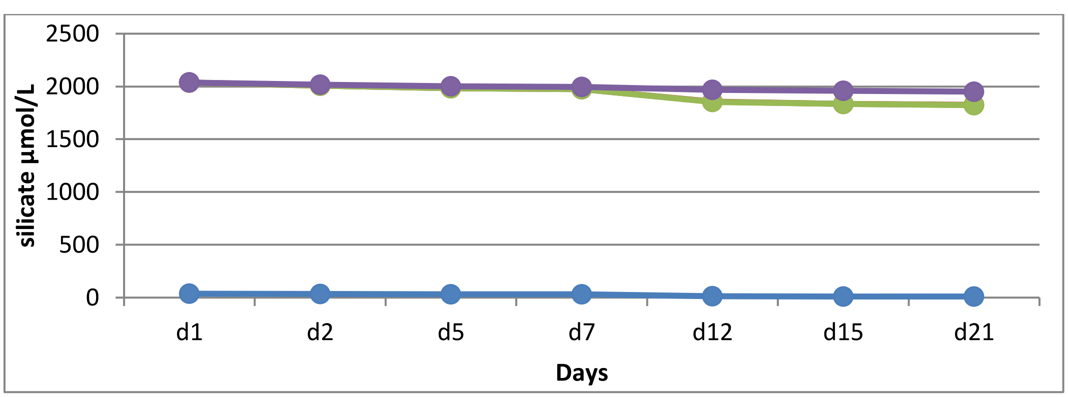


Figure 1: Time evolution of the concentrations of phosphates, nitrates, silicates, and ammonia within treatments (control—, Fe10—, Fe100—, nutrients—) **for the experiments carried out at the river al Kabir al Shamali Estuary station (K).**







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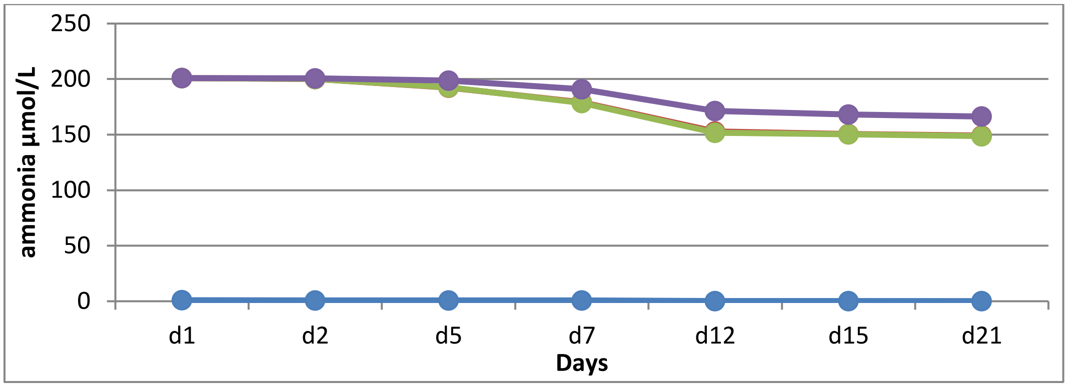

Figure 2: Time evolution of the concentrations of phosphate, nitrate, silicates, and ammonia within the treatments (control—, Fe10—, Fe100—, nutrients—) **for the experiments conducted at the Marine Research Station (M).**

The concentrations of chlorophyll a varied for the studied sites, where the concentrations of chlorophyll a were in the downstream region on the first day of incubation and before adding nutrients and Fe (1.038 mg.m^{-3}) While I reached in the area (m) 0.091mg.m^{-3} (Table 1).

This discrepancy is attributed to the spatial differences of the studied areas, where the downstream area (K) is considered a region rich in nutrients coming to it from the waters of the river that passes through the agricultural lands and transports these materials to the downstream region, while the region (M) is considered a semi-closed region due to the lack of external inputs for these Region (Krawi et al., 2018, Jolak et al., 2013, Darwich and Sulaiman, 2013).

We did not observe an increase in phytoplankton growth on the second day of the experiment in the four preparations, due to the adaptation of the phytoplankton to the new source of nutrients, the intensity of exposure to light and temperature. A previous study reported that these factors were taken into account, as the level of chlorophyll in phytoplankton is sufficiently sensitive to any environmental changes (Mohamed and Amil, 2015).

Phytoplankton started to grow clearly in all mediums, starting from the fifth day of incubation, as the graph of chlorophyll a started to rise, forming the beginning of the phytoplankton growth jump (Figures 3, 4). Observe the low growth rates at the marine research station due to the fact that the initial concentrations of nutrients and chlorophyll a are low (Table 1).

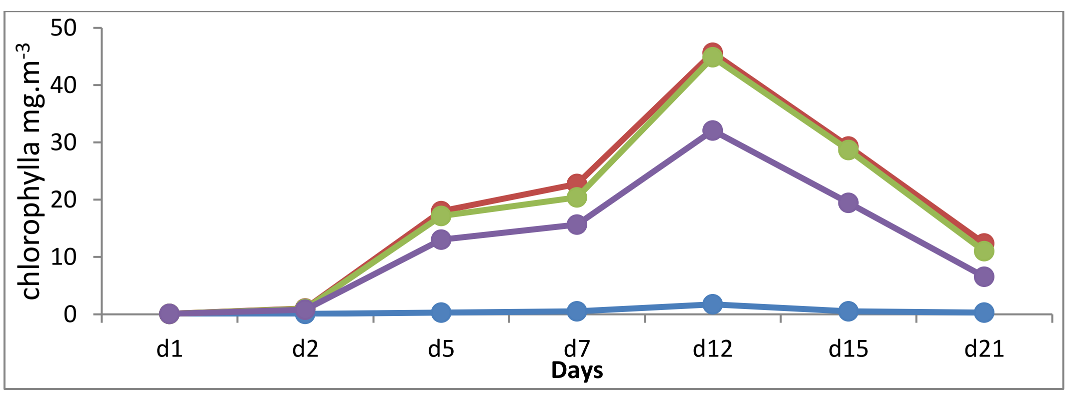

Figure (3): Time evolution of chlorophyll a concentrations for the experiments carried out within the preparations (control—, Fe10—, Fe100—, nutrients—) for the experiments carried out at the Marine Research Station (M).

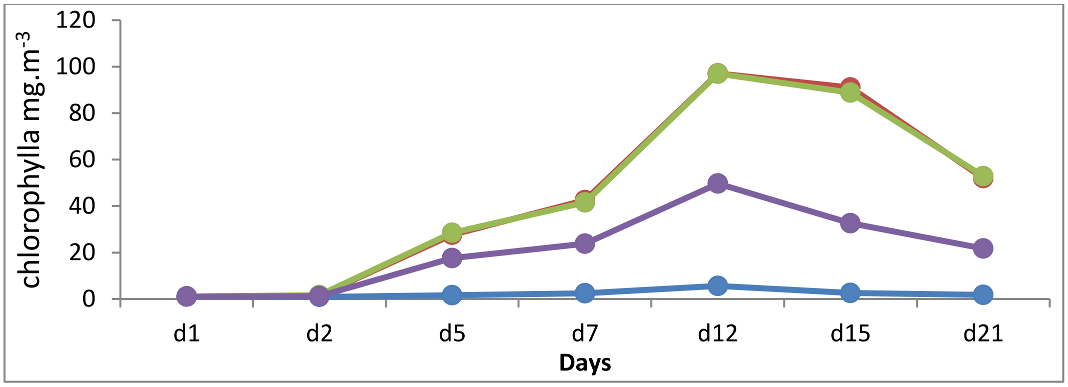

Figure (4): Time evolution of chlorophyll a concentrations for the experiments carried out within the preparations (control—, Fe10—, Fe100—, nutrients—) for the experiments carried out at the river al Kabir al Shamali Estuary (K).

A jump occurred in the concentrations of chlorophyll a on the twelfth day (d_{12}) in all the preparations and it formed a clear peak as indicated (Figures 3, 4). The concentrations of chlorophyll a at station M reached the highest value of Max Chl a on the twelfth day of incubation recorded (97.063 $mg.m^{-3}$) In the second treatment (Fe-10) Then the concentrations of chlorophyll a began to decline after the twelfth day of incubation.

The concentrations of chlorophyll a reached during the peak on the 12th day (d_{12}) of the marine research area in the control sample (1.732 $mg.m^{-3}$) In the second treatment (45.637 $mg.m^{-3}$), In the third treatment (44.863 $mg.m^{-3}$) In the fourth treatment (32.092 $mg.m^{-3}$) (Table2) Where the maximum increase was in the second preparation and amounted to about 26 times." No significant difference was observed in the concentration of chlorophyll a between the second and third treatments, that is, adding Fe in a concentration. 10 μmol/L Sufficient for chlorophyll a to reach its maximum value in the studied treatments. The increase in the fourth treatment was about 15 times, which confirms that our water is limited in nutrients and less than Fe, but the addition of Fe promotes the growth of phytoplankton and thus chlorophyll a.

The same is the case in the estuary site, where the results indicated an increase in chlorophyll a concentrations greater than in area (m). This can be explained by the fact that the primitive biomass (the concentration of chlorophyll a at the start of the experiment) was greater than it is in station (M) (Table 1), and that the nature of the phytoplankton in the downstream area (K) is adapted to the high concentrations of nutrients, where the concentrations of chlorophyll a reached during Peak (12th day of the experiment) in the downstream region for the control samples (5.631 $mg.m^{-3}$) .

The second treatment (97.063 $mg.m^{-3}$) And the third treatment (96.946 $mg.m^{-3}$) And the fourth treatment (49.642 $mg.m^{-3}$), The maximum increase was in the second report and reached 17 times compared to the control sample, then the third report and to a lesser degree the fourth report, which amounted to 9 times.

The amount of growth of phytoplankton in the downstream area (k) was greater compared to the research area (m), but it is less for the doubling from the initial concentration of chlorophyll a because the amount of external supply of Fe and other trace minerals in the downstream area is greater than it is in the research area The marine constituting a semi-enclosed area (Krawi et al., 2018.)

As a result, adding Fe led to an increase in the nutrient consumption rates in both locations compared to the fourth preparation (nutrients), which contains nutrients without Fe (Table 2). The consumption of nutrients was also accompanied by an increase in chlorophyll a concentrations, and the growth continued throughout the experiment period and was accompanied by a jump in consumption on the twelfth day of the experiment. We also noticed only slight differences in the consumption of nutrients between the concentrations of Fe 10 and 100, which indicates that the amount of Fe at concentration 10 was sufficient to raise the level of chlorophyll a to its maximum value in the studied samples. These results are consistent with the results of research that dealt with adding iron to improve primary productivity in marine waters (Balaguer et al., 2022, Mohamed and Amil, 2015, Anderson et al., 1982, Boye and van den Berg, 2000, Doucette and Harrison, 1991).

4. CONCLUSION

The initial concentrations of chlorophyll a and the nutrient electrolytes in the Al Kabeer Al Shamali estuary region recorded higher values than those recorded in the marine research area. Phytoplankton showed a clear growth ability in the two studied areas in the enrichment media, as growth continued throughout the experiment period and nutrients remained available in the medium until the end of the experiment. The jump in growth occurred on the twelfth day of the experiment in all treatments, while the growth of

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phytoplankton was less in the control sample and growth stopped on the twelfth day of the experiment in area (m) as a result of phosphate depletion from the medium. Iron played an important role in stimulating growth, as the results showed that iron fortification treatments recorded the highest values for phytoplankton growth and consumption of nutrient electrolytes compared to the rest of the treatments. The concentration of iron 10 μmol/L is sufficient to reach a maximum growth of phytoplankton, as no clear differences were observed in growth compared to the concentration of 100 μmol/L.

RECOMMENDATIONS

1. Continuing such experiments on different areas of the Syrian coast
2. Working on the application of experiments directly on the site to determine the role of Fe in affecting the primary productivity in our region.
3. The possibility of investing these results economically in fish farms and relying on phytoplankton that stimulated their growth with Fe as an alternative to manufactured or manually compounded feed.
4. Using the results of this study in the environmental field to reduce global warming through the process of photosynthesis that consumes carbon dioxide.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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